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2976-Pos Board B668

Interprotein Electron Transfer with Protein Maquettes

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Protein-driven electron transfers are essential steps in a wide range of biochemical processes. Electron transfer rates are governed primarily by the distance between redox centers and by the driving force originating from midpoint potentials and coupled catalytic reactions. The structural complexity of natural proteins contrasts with the relatively simple rules of cofactor selection and placement that, in principle, govern the electron transfer behavior. Here we present two examples of minimal 4-α-helical bundle proteins ("maquettes") that leverage these basic design principles to illustrate functional electron transfer. Both proteins support bis-histidine ligation of natural heme cofactors within a tetrahelical bundle. The first of these maquettes is BT6, a water-soluble monomeric protein with a net charge of −14. Reduced heme-bound BT6 transfers electrons to cytochrome c at $2x10^7$ M/s, a rate similar to the electron transfer between the natural *b*-heme protein neuroglobin and cytochrome *c* during apoptosis. As in the neuroglobin-cyt *c* system, we believe that the rapid electron transfer from glutamate-rich BT6 to lysine-rich cytochrome *c* is enhanced through electrostatic interaction between the two proteins. The second maquette discussed here is AM1, an amphiphilic 4-α-helical monomer. AM1 incorporates three bis-histidine binding sites to ligate a chain of heme cofactors spanning a phospholipid bilayer. We performed stopped flow experiments to probe transmembrane electron transfer, mixing natural cytochrome *c* with AM1 liposomes encapsulating photoreduced flavin mononucleotide. In the presence of protein and heme, transmembrane electron transfer rates are significantly faster than in the absence of either.

2977-Pos Board B669

Quantum Delocalization of Protons in the Ketosteroid Isomerase Active Site

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Ketosteroid isomerase (KSI) catalyzes steroid isomerization with extremely high efficiency and has become a paradigm of enzymatic proton transfer chemistry. In this poster I will show our recent research which has identified how the hydrogen bond network formed by this enzyme facilitate proton delocalization and sharing in the active site. This quantum delocalization greatly stabilizes a deprotonated tyrosine residue in the active site and leads to a 10,000 fold increase in its acid dissociation constant. This study is made possible by a series of technical breakthroughs developed in our group that greatly enhance the efficiency of including quantum mechanical fluctuations in simulating condensed phase systems and extracting isotope effects. These advances allow us to investigate the quantum delocalization of protons in the active site of KSI, which might shed light on the origin of the enzyme's remarkable catalytic efficiency and guide the development of synthetic catalysts.

2978-Pos Board B670

Femtosecond 2D-IR Spectroelectrochemistry of Biomolecules

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Electron transfer reactions are a major class of reactions occurring in biological systems, and at the heart of photosynthesis as well as cellular respiration. The understanding of the electron transfer reactions is thus a pivotal task on the way to gain insight into the complex electron transfer mechanism in some major proteins such as terminal oxidases. Various electrochemical and spectroscopic techniques are currently used in order to elucidate electron transfer reactions in proteins and spectroelectrochemical techniques have the main asset of simultaneously probing the electrochemical and the spectroscopic properties of proteins during the course of electron transfer (1, 2).
In this contribution we demonstrate the combination of spectroelectrochemistry with ultrafast 2D-IR spectroscopy, a powerful technique for the investigation of structural dynamics (3). This combination gives direct structural information by the measured anharmonicities, transition dipole angles and couplings of systems that undergo electron transfer in general and in biological systems in particular. Using an electrochemical cell designed for femtosecond spectroscopy, it becomes possible to trigger an electrochemical reaction in-situ and to measure the ultrafast dynamics of the different redox states of a sample under direct electrochemical control, making thus the various redox states of the super-family of redox active proteins accessible.
The first redox-coupled 2D IR spectra are reported here for a sample of biological relevance, riboflavin which is the prosthetic group of electron-transfer flavoproteins. The ultrafast dynamics as well as the structural changes detected upon electron transfer reactions will be discussed.

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2979-Pos Board B671

Modeling H-Bonding and Protonation States of Membrane Buried ARG-TYR Pairs

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Previous X-ray crystallographic studies have shown that in the activated states of G-protein coupled receptors (GPCRs), or in those of microbial rhodopsins, the side chains in conserved R+Y dyads transiently move within several Å of each other. In the M state of bR, substantial side-chain perturbation occurs, as has been shown previously, e.g. by FTIR and solid-state (SS) NMR studies of bR with ^{15}N-labeled R82. No simpler system has been able to model the transient spectral perturbations of R82 in M. Here we report the synthesis of crystalline ω-(4-anisolyl)-dodecylguanidine (ADG) and ω-(4-phenolyl)-dodecylguanidine (PDG), with either natural isotope abundance or 98% ^{15}N-enrichment at the 2 terminal nitrogens. The ADG free base, with a deprotonated guanidine group, is a good spectroscopic model for both IR and ^{15}N-SS-NMR signals that were previously seen from R82 in the M photointermediate, particularly for the unusually large ^{15}N chemical shift splitting. In the crystalline PDG free base, however, this chemical shift splitting is reduced to nearly the same value seen for the HBr salts of ADG, PDG, and arginine itself, all of which have protonated guanidinium groups. The high-resolution X-ray structure of the free-base form of PDG, as well as the IR spectrum of these crystals in KBr disk, show clearly that what crystallizes is actually a zwitterion, with guanidinium and phenolate groups; additionally, 2 H-bonding solvent (methanol) molecules co-crystallize with each PDG. These PDG free-base crystals are surprisingly insoluble in most aprotic solvents. However, they are sufficiently soluble in DMSO to observe IR, UV-vis and ^{1}H NMR spectra. These spectra are all consistent with a ~50/50 mix of rapidly-interchanging zwitterionic and neutral forms. The high proton polarizability of PDG makes this an interesting model system for buried H-bonded uncharged R+Y dyads in proteins.

2980-Pos Board B672

Roles for Protons in Living Membranes: Cardiolipin

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Cardiolipin (CL) in mitochondria has 2 unique headgroup conformations. In lipid bilayers (pK >7.0) it traps 2 protons forming patches. It also binds supercomplexes together where its phosphates bind cations. Patches trap protons with short, strong H-bonds (SSHBs) as described by Al Mildvan (Meth Enz 2007). CL's SSHBs trap protons its phosphates. They're tightly held due to insertion of CL's 4 chains into the bilayer. The H^+, not available to water, has no affect the pH. Yet patches may act as a H^+ antenna, pK >7.0, on both sides of the IM. X. Periole (JACS & Nature 2013) has identified CL-patch-binding-sites on the Xray structures of cyt bc1 & cyt oxidase, near the entrance to their putative H^+ channels. Thus H^+ delivery is on a microsecond (μs) timescale, like the chloroplast's chlorophyll antenna. The complexes pump on a msec timescale. CL patches are on the matrix side are near the F0 of the F0, F1 ATPase, the protons would charge F0 of the F0, F1, ATPase. SSHB formation minimally changes the pH on both membrane faces. Pumped protons have little ΔpH but a maximum ΔΨ. Junge & Oster have each shown that the F0 protons are driven by ΔΨ, not ΔpH.
Living membrane surfaces are polyanionic, mostly on the lipid headgroups, tightly held together by their chains. Guoy-Chapman tells us that this decreases the surface pH. SHHB trapped protons have no effect on the pH. Finally, the ΔΨ of most living membranes is created by proton pumps on a msec timescale, but is adjusted by K-channels on a μs timescale. ΔΨ provokes wasteful leakage of H^+s across the membrane. ATP energy pumps them back! Lipids, polyisoprenes, sterols, iso- and anteiso- chains, hopanoids and squalene inhibit this leakage.